Performance Assessment of Sysmex® CA-104 for Screening Coagulogram Testing

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ABSTRACT

Objective: The Sysmex® CA-104 (CA-104) is a new, semi-automated coagulation analyzer which has not yet been evaluated in Thailand. The objective of this study was to evaluate the CA-104 performance for prothrombin time (PT), activated partial thromboplastin time (APTT), and international normalized ratio (INR) under local laboratory conditions.

Methods: Real patient samples and control materials were used to evaluate CA-104 in terms of precision, reference range, and comparability to the validated Sysmex® CS-2100i analyzer (CS-2100i).

Results: The highest percentages of coefficient of variation of PT and APTT were 1.34 and 1.47 for within-run, and 3.2 and 1.65 for between-run precision studies. Correlation coefficients of PT, APTT, and INR between CA-104 and CS-2100i were 0.97, 0.95, and 0.99, respectively. Mean biases of PT, APTT, and INR of CA-104 compared with those from CS-2100i were low. Most results were within 95% agreement, although a few values at the high levels were not. For INR testing, no specimen required warfarin dose adjustment when using CA-104 instead of CS-2100i.

Conclusion: Performance of CA-104 for screening coagulogram in terms of precision and comparability to CS-2100i was acceptable. However, a few discrepancies in clotting time at high values were observed. Further investigation to identify the cause of this discrepancy is warranted.

Keywords: Sysmex® CA-104, automated analyzer, performance assessment, screening coagulogram

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INTRODUCTION

Screening coagulogram is a group of tests which are collectively used to assess bleeding risk. It is generally composed of prothrombin time (PT) and activated partial thromboplastin time (APTT).[1] In some laboratories, thrombin time and fibrinogen assay are also included in the screening coagulogram. There are two methods of screening coagulogram; the traditional manual tilt-tube method and semi or fully-automated analyzer. Most laboratories now use automated analyzers, because of increased efficiency in an increasing work load environment. Automated analyzers are based on one of two different principles; electro-mechanical or photo-optical.[2,3] Each principle has its own advantages and disadvantages.[2] The decision to implement a new analyzer usually depends on the performance of the analyzer. It is recommended that each laboratory evaluate analyzer performance to assure appropriate function under local laboratory environment before full implementation.[4]

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Sysmex® CA-104 (Sysmex Corporation, Kobe, HYG, Japan) or CA-104 is a new, semi-automated coagulation analyzer which utilizes turbodensitometric measuring principle, which is suitable for small laboratories or clinics. It can also be used as a backup option for the fully-automated analyzer. However, CA-104 is a new product on the market and no study regarding its performance has been conducted in any local laboratory. Accordingly, the objective of this study was to validate the performance of CA-104 in screening coagulogram testing, including PT and APTT, under local laboratory conditions.

MATERIALS AND METHODS

Analyzers and reagents

The analyzer being evaluated was CA-104. The principle of this analyzer is based on turbodensitometric measurement, which constitutes two features: stirring action and optical detection. The stirrer bar in the reaction cuvette mixes the reagent and plasma and then a small whirl of mixture is created to ensure that a fibrin clot is formed in front of the photo detector. Light with a wavelength of 870 nm then passes through the cuvette. Clotting time is recorded when transmitted light intensity decreases from the initial level to the set point due to fibrin formation.

CA-104 was compared with the previously validated Sysmex® CS-2100i analyzer (Sysmex Corporation, Kobe, HYG, Japan) from a comparison study.[5] The reagents used for PT and APTT testing in the CA-104 and CS-2100i systems were Thromborel® S, Lot 5545538 and Dade® Actin FS, Lot 538466, respectively (Siemens Healthcare Diagnostics, Marburg, Germany). The CS-2100i was regularly checked for quality and reliability by both internal quality controls and external quality assurance scheme.

Performance evaluation

Performance evaluation included precision studies, reference range determination, and comparison studies. Blood specimens used in these studies, except for the precision study which used quality control materials, were plasmas collected in 3.2% sodium citrate tubes. The evaluation protocol was part of a previously recommended validation method.[6,7]

Precision study

Forty quality control materials, comprised 20 control plasma level 1 (Lot 178292) and 20 control plasma level 2 (Lot 278292) (Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used. Control materials were analyzed repeatedly for PT and APTT in a single run for 20 measurements (within-run precision) and in the morning and evening over a period of 10 consecutive days (between-run precision). Percentages of coefficient of variation (%CV) were then calculated.

Reference range determination

Specimens from 44 normal subjects from the annual health checkup clinic were tested for PT and APTT by CA-104 over a period of several days. Subject age varied from 23 to 57 years old. Exclusion criteria included: history of bleeding or thrombotic disorders, acute illness, pregnancy, hormone contraceptive use, and any taking of medication. Mean ± 2 times standard deviation (S.D.) and values between 2.5^{th} and 97.5^{th} percentiles were designated as reference ranges for the normally distributed and non-normally distributed data, respectively. Outliers, which were defined as values whose difference from the closest value was more than 0.33 of the range, were excluded.[7]

Comparison study

Forty specimens from non-anticoagulated patients, as well as 55 specimens from patients receiving long-term warfarin therapy were tested for PT and APTT by CA-104 and CS-2100i simultaneously over a period of several days. The specific geometric mean of PT and international sensitivity index (ISI) of each system were used to calculate the international normalized ratio (INR) for specimens from patients receiving warfarin therapy. Correlation and agreement between analyzers were then demonstrated by correlation coefficient (r^2) and Bland-Altman plot for each test parameter.

Clinical agreement between methods regarding decision making for warfarin dose adjustment was also analyzed. Given that the

therapeutic range is INR by the CS-2100i of 2 to 3 units and the trigger level of dose adjustment is the INR outside the therapeutic range of ± 0.2 INR units, and the numbers of specimens that would affect dosage changes when using CA-104 were counted.[8]

Statistical analysis

Microsoft Office Excel version 2010 (Microsoft Corporation, Redmond, WA, USA) was used to calculate mean, standard deviation (S.D.), and %CV. Excel was also used to prepare linear regression lines, r^2, and Bland-Altman plots for comparability determination. The SPSS software version 16.0 for Windows (SPSS, Inc., Chicago, IL, USA) was used for normality testing of the distribution (Kolmogorov–Smirnov test) for reference range determination.

Ethical consideration

This study was approved by the Siriraj Institutional Review Board (SIRB Protocol No. 751/2557).

RESULTS

Precision study

Means, S.D., and %CV from within-run and between-run studies are presented in Table 1.

Reference range determination

No statistical outliers were observed in this study. PT results from normal subjects were normally distributed, while APTT results were not. Reference ranges for PT and APTT were 9.2 to 12.3 seconds and 23.1 to 30.7 seconds, respectively. Both of these ranges were within the ranges recommended by the manufacturer.

Comparison study

Linear regression lines and r^2 of PT, APTT, and INR between CA-104 and CS-2100i are described in Fig 1. PT, APTT, and INR values covered the clinical significance ranges. The r^2 were 0.97, 0.95, and 0.99 for PT, APTT, and INR, respectively. The related Bland-Altman plots are shown in Fig 2.

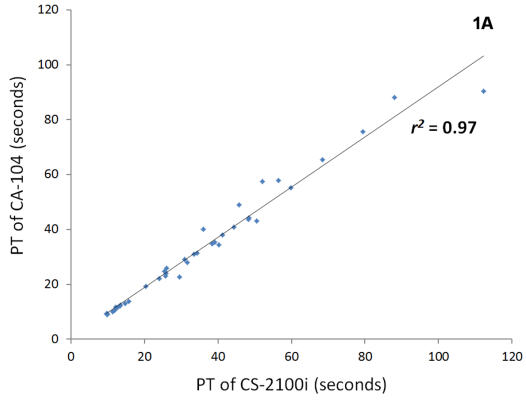
To determine clinically relevant agreement regarding warfarin monitoring, specimens with INR ranging from 1.01 to 9.7 by CS-2100i were included. Regarding INR from CA-104, there was no discordance of results that affected warfarin dose adjustment.

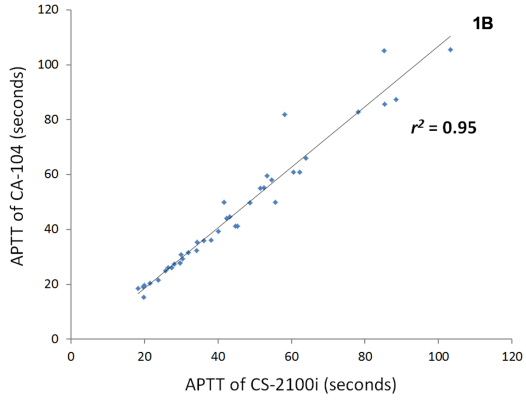
DISCUSSION

In this study, the authors evaluated the performance of the CA-104 analyzer in terms of precision and comparability to the validated CS-2100i analyzer. For precision assessment, it is recommended that within-run and between-run %CVs of the assays should not be more than 25% and 33%, respectively, of allowable total error, as defined by the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88).[9] The %CVs of CA-104 were less than those values, i.e. 3.75% for PT and APTT for within-run precision and 5% for PT and APTT for between-run precision. These findings were found in both normal and abnormal levels of control materials. Therefore, the precision of CA-104 was found to be acceptable for PT and APTT.

TABLE 1. Mean, standard deviation (SD), and percentage of coefficient of variation (%CV) of prothrombin time (PT) and activated partial thromboplastin time (APTT).

	PT				APTT			
	Within-run		Between-run		Within-run		Between-run	
	Control Plasma Level 1	Control Plasma Level 2	Control Plasma Level 1	Control Plasma Level 2	Control Plasma Level 1	Control Plasma Level 2	Control Plasma Level 1	Control Plasma Level 2
Mean	10.70	38.28	10.96	40.89	27.22	68.90	27.74	70.68
SD	0.09	0.51	0.15	1.31	0.20	1.02	0.34	1.16
%CV	0.83	1.34	1.37	3.20	0.72	1.47	1.21	1.65





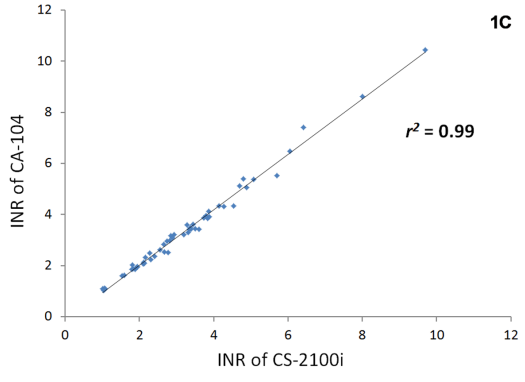
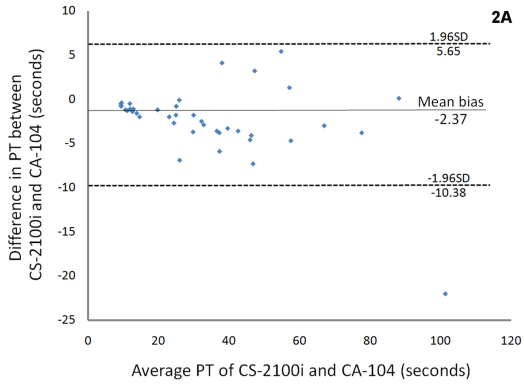
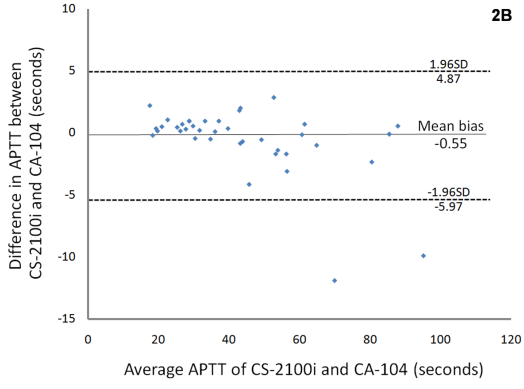


Fig 1. Linear regression lines and correlation coefficients (r^2) for prothrombin time (PT), activated partial thromboplastin time (APTT), and international normalized ratio (INR), as determined by Sysmex® CA-104 (CA-104) and Sysmex® CS-2100i (CS-2100i). Figures 1A, 1B, and 1C for PT, APTT, and INR, respectively.





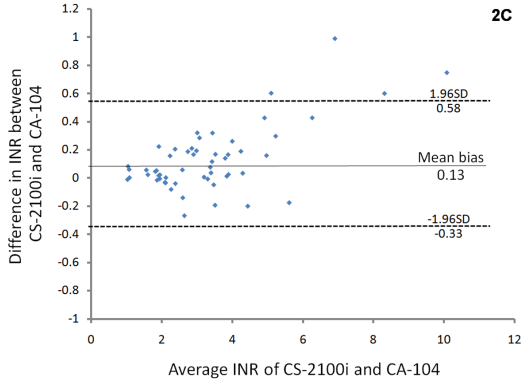


Fig 2. Bland-Altman plots of differences in prothrombin time (PT), activated partial thromboplastin time (APTT), and international normalized ratio (INR) as determined by Sysmex® CA-104 and Sysmex® CS-2100i. Figures 2A, 2B, and 2C for PT, APTT, and INR, respectively.

It is recommended that each laboratory establish its own reference range, specific to the types of analyzer and reagent.[6,7] Reference ranges for PT and APTT for CA-104 determined in this study were derived from the local population. As such, this information is transferable to small laboratories that use the same analytical system for routine use. However, it should be emphasized that these ranges are specific to the reagent lot. Laboratories have to establish new values when they change reagent lot.

The PT, APTT, and INR of CA-104 were compared to those of the validated CS-2100i system. Values of selected specimens in this study covered the clinical significant ranges, varying from low to high levels. Correlations of PT,

APTT, and INR were excellent, as demonstrated by the r^2 of 0.97, 0.95, and 0.99, respectively. For agreement analysis by Bland-Altman plot, the mean biases of PT, APTT, and INR of CA-104 compared with those from CS-2100i were low. The highest bias from CA-104 was found in PT testing (-2.37 seconds), which may not be clinically significant. For INR monitoring, there was no significant change in warfarin dose adjustment when using CA-104, instead of CS-2100i. Most results for PT, APTT, and INR were within 95% agreement, although a few values at the high levels were not. These discrepancies in clotting time may be explained by differences in detection principle and/or the analytical process. Clotting time in CS-2100i is calculated from the middle of the coagulation curve, while clotting time in the CA-104 is reported when the instrument detects a fixed decrease in transmitted light intensity caused by the fibrin whirl. Specimens with normal clotting time usually generate a strong fibrin whirl, which can decrease the transmitted light earlier. However, a specimen with prolonged clotting time may have a weaker fibrin whirl. As a result, a longer time may be needed to develop a strong fibrin for the analyzer to detect. This effect may cause discrepancies between CA-104 and CS-2100i at high levels of clotting time. Additional testing on discrepant specimens to prove this proposed cause (e.g., fibrinogen assay) is warranted. In addition, CA-104 is a semi-automated analyzer in which accuracy and precision of testing are dependent on the operator. Inter-operator variation and competency of operator can affect the accuracy of test results. However, this would not explain discrepant results in this study, because all assays were performed by a well-trained investigator and the precision of all parameters was within acceptable limits.

In conclusion, CA-104 is a new, semi-automated coagulation analyzer that can perform screening coagulogram assays. Performance of CA-104 in terms of precision was found to be acceptable. Comparability of PT, APTT, and INR between CA-104 and CS-2100i were acceptable. However, a few discrepancies in clotting time at high values were observed. While this discrepancy would not change a patient's diagnosis, it could potentially affect follow-up. Further investigation to identify the cause of this discrepancy at high values is warranted. Furthermore, other aspects about the performance of CA-104 should also be investigated, e.g. effect of interferences and comparison of APTT ratio in the heparinized patients.

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